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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/079,134	02/20/2002	Theo T. Nikiforov	100/14010	7013

21569 7590 08/08/2003

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EXAMINER
GOLDBERG, JEANINE ANNE

ART UNIT	PAPER NUMBER
1634	

DATE MAILED: 08/08/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/079,134	NIKIFOROV, THEO T.	
	Examiner	Art Unit	
	Jeanine A Goldberg	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *27 May 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disp sition of Claims

4)☒ Claim(s) *1-18* is/are pending in the application.

4a) Of the above claim(s) *14-18* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *02 February 2002* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *0602* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

1. This action is in response to the papers filed May 27, 2003. Currently, claims 1-18 are pending. Claims 14-18 have been withdrawn as drawn to non-elected subject matter.

Election/Restrictions

2. Applicant's election without traverse of Group I (Claims 1-13) in the paper filed May 27, 2003 is acknowledged.

Priority

3. This application claims priority to provisional application 60/270,67, filed February 22, 2001.

Drawings

4. The drawings are acceptable.

Specification

5. The disclosure is objected to because of the following informalities. The specification refers to a 3'-5' polymerase citing preferred DNA polymerases as Taq polymerase, Thermosequenase etc. Each of these polymerases are 5'-3' rather than 3'-5'. Appropriate correction is required.

Claim Objections

6. Claim 9 is objected to because of the following informalities.

The claim is directed to an "exonucleoase". Exonuclease appears to be misspelled. Appropriate correction is required.

Claim Rejections - 35 USC § 112- Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

7. Claim 4 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

A) Claim 4 is indefinite over the recitation 3'-5' DNA polymerase enzyme. DNA polymerase enzymes polymerize in the 5'-3' direction. The specification refers to a 3'-5' polymerase citing preferred DNA polymerases as Taq polymerase, Thermosequenase etc. Each of these polymerases are 5'-3' rather than 3'-5'. Therefore, it is unclear whether the claims are drawn to 3'-5' DNA polymerases, or whether the claims are drawn to examples provided in the specification of 5'-3' DNA polymerase enzymes.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

8. Claims 1-2, 4-9, 12-13 rejected under 35 U.S.C. 102(b) as being anticipated by Wallace et al. (US Pat. 5,639,611, June 1997).

Wallace et al. (herein referred to as Wallace) teaches a method of allele specific polymerase chain reaction. Wallace teaches the method is rapid, non-radioactive method for allele specific detection in which the 3' terminal nucleotide of one of the primers of the primer set forms a match with one allele and a mismatch with the other allele. Wallace exemplifies using primers to the human beta-globin gene. DNA from individuals containing beta-globin was analyzed. Wallace uses oligonucleotide sequence. The oligonucleotides HB14A and HB14S, which are 14 nucleotides in length, differ only in the final 3' nucleotides, such that the oligonucleotides contain a subsequence and a terminal nucleotide which is complementary to one of the possible variant nucleotides (limitations of Claim 2, 6-7). Wallace teaches using labeled oligonucleotides. One of the oligonucleotide primers is labeled at the 5' end with a fluorescent group and the other primer is labeled with biotin (col. 3, lines 15-20; col. 5, lines 15-25, claim 4). With respect to claim 5, as written the claim requires that the first fluorescent label is coupled to the first terminal nucleotide. Wallace teaches coupling the label to the 5' end which is a terminal nucleotide. The fluorescent group is suitable for measuring the amount of the fragment produced. Wallace teaches performing the reaction with first and second primer sets which are differentially labeled at the 5' end with a different fluorescent group (limitations of Claim 12). The oligonucleotides are contacted and allowed to react in the presence of nucleotides, template and Thermus aquaticus DNA polymerase (col. 4, claim 2)(limitations of Claim 4, 8-9). Wallace

teaches that the reaction products are quantified by measuring the fluorescence (col. 6, claim 3). The method allows direct detection without probe hybridization, ligation or restriction enzyme cleavage. Therefore, since Wallace teaches every limitation of the instant claims, Wallace anticipates the claimed invention.

9. Claims 1-2, 4-9, 12-13 rejected under 35 U.S.C. 102(a) as being anticipated by See et al. (BioTechniques, Vol. 28, No. 4, pages 710-716, April 2000).

See et al. (herein referred to as See) teaches a method of detecting single nucleotide polymorphisms (SNPs) using primers labeled with fluorescent dyes to rapidly and accurately differentiate among alleles that are defined by a single nucleotide difference (abstract). Specifically, See teaches a method of identifying a nucleotide in at least a first position, namely a SNP; hybridizing an oligonucleotide to the target sequence; wherein the oligonucleotide comprising nucleotides immediately adjacent to the first position, a terminal nucleotide and a florescent label; performing a extension reaction with polymerase extension reagents; monitoring a fluorescent signal; and identifying the first position. Specifically, See teaches a method which depends on pre-amplification and the use of two differently labeled allele specific primers per locus, each of which matches one of two possible allele state in the 3' nucleotide (page 710, col. 3)(limitations of Claim 2). Following initial amplification, labeled internal primers were added to the amplification mixture along with fresh Taq DNA polymerase, dNTPs and buffer (limitations of Claim 4, 8-9). The fluorescent products were amplified and then resolved on gels for detection (page 711, col. 1). The method characterizes the

products both by size and by fluorescent label which permits efficient multilocus analysis (page 711, col. 1)(limitations of Claim 12-13). As seen in Table 1, the internal SNP primers each contain a fluorescent dye on the 5' end of the primer (limitations of Claim 5). With respect to claim 5, as written the claim requires that the first fluorescent label is coupled to the first terminal nucleotide. See teaches coupling the label to the 5' end which is a terminal nucleotide. The internal primers are 20 nucleotides in length (limitations of Claim 6-7). Since See teaches every limitation of the instant claims, See anticipates the claimed invention.

10. Claims 1-2, 4, 6-9, 13 are rejected under 35 U.S.C. 102(b) as being anticipated by Chen et al. (PNAS, Vol. 94, pages 10756-10761, September 1997) as evidenced by Newton et al. (Nucleic Acids Research, Vol. 17, no. 7, 2503-2515, 1989).

Chen et al. (herein referred to as Chen) teaches a method of fluorescence energy transfer detection as a homogeneous DNA diagnostic method. The method of template-directed dye-terminator incorporation (TDI) assay, has been developed for mutation detection and high throughput genome analysis. The method starts with total human DNA, amplified by PCR, and a primer extension reaction is performed (abstract). As seen in Figure 1, the TDI assay is illustrated. Dye-primer, allele specific dye-terminators are added with polymerase. Depending on the nucleotide incorporated, the fluorescent signal will differ (page 10757). Chen teaches using AmpiTaq DNA polymerase (page 10757, col. 2)(limitations of Claim 4). The fluorescence intensities were analyzed and plotted (see Figure 2).

As seen in Figure 1, the method comprises identifying a nucleotide in at least a first position in a polynucleotide, namely the nucleotides at all positions within the primer and a position 3' of the primer. Chen teaches providing a genomic template, hybridizing a probe which comprises a subsequence of nucleotides, a first terminal nucleotide and a first florescent label (see step 4, Figure 1). The primer comprises a terminal nucleotide complementary to one possible nucleotide in the position, namely a nucleotide complementary. The probe and target are contacted with allele- specific dye-terminators and DNA polymerase, namely AmpliTaq DNA polymerase (limitations of Claims 4, 8-9). The changes in fluorescence intensities is monitored which is indicative of the presence of polymerase extension of the probe, such that the presence of the polymerase extension of the probe indicates that the terminal nucleotide is complementary to the nucleotide in the first position. Given the properties of Taq polymerase and the lack of its ability to function in PCR reactions in the presence of a 3'-residue mismatch, the fact that the primer of Chen is extended suggests that the terminal position is properly matched, thereby identifying the first position as complementary to the terminal nucleotide (see Newton, abstract). The method of Chen further includes steps to identify the nucleotide 3' of the primer. In the example provided by Chen, the subsequence would comprise ...AGTG. Chen also gives specific primers for CF508; HC282Y and MENC634F which are 25 nucleotides in length (limitations of Claims 6-7). The florescent label is Fluorescein, attached to the 5' end. And the terminal nucleotide is A (limitations of Claim 2). The polymerase extension is performed to add an allele-specific dye-terminator. The fluorescent signal from the

reaction is monitored and indicates the presence of extension of the probe to identify not only the positions within the primer, but also the position of the nucleotide immediately 3' of the hybridized primer. Chen additionally teaches performing a amplification prior to the detection method (see Figure 1, step 1)(limitations of Claim 13). Thus, as written, Chen teaches every limitation of the instant claims, therefore, Chen anticipates the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

11. Claim 3 is rejected under 35 U.S.C. 103(a) as being unpatentable over Wallace et al. (US Pat. 5,639,611, June 1997) or See et al. (BioTechniques, Vol. 28, No. 4, pages 710-716, April 2000) in view of Markiewicz et al. (Nucleic Acids Research, Vol. 25, No. 18, pages 3672-3680, 1997).

Wallace et al. (herein referred to as Wallace) teaches a method of allele specific polymerase chain reaction. Wallace teaches the method is rapid, non-radioactive method for allele specific detection in which the 3' terminal nucleotide of one of the primers of the primer set forms a match with one allele and a mismatch with the other allele. Wallace exemplifies using primers to the human beta-globin gene. DNA from individuals containing beta-globin was analyzed. Wallace uses oligonucleotide

sequence. The oligonucleotides HB14A and HB14S, which are 14 nucleotides in length, differ only in the final 3' nucleotides, such that the oligonucleotides contain a subsequence and a terminal nucleotide which is complementary to one of the possible variant nucleotides (limitations of Claim 2, 6-7). Wallace teaches using labeled oligonucleotides. One of the oligonucleotide primers is labeled at the 5' end with a fluorescent group and the other primer is labeled with biotin (col. 3, lines 15-20; col. 5, lines 15-25, claim 4). With respect to claim 5, as written the claim requires that the first fluorescent label is coupled to the first terminal nucleotide. Wallace teaches coupling the label to the 5' end which is a terminal nucleotide. The fluorescent group is suitable for measuring the amount of the fragment produced. Wallace teaches performing the reaction with first and second primer sets which are differentially labeled at the 5' end with a different fluorescent group (limitations of Claim 12). The oligonucleotides are contacted and allowed to react in the presence of nucleotides, template and Thermus aquaticus DNA polymerase (col. 4, claim 2)(limitations of Claim 4, 8-9). Wallace teaches that the reaction products are quantified by measuring the fluorescence (col. 6, claim 3). The method allows direct detection without probe hybridization, ligation or restriction enzyme cleavage.

See et al. (herein referred to as See) teaches a method of detecting single nucleotide polymorphisms (SNPs) using primers labeled with fluorescent dyes to rapidly and accurately differentiate among alleles that are defined by a single nucleotide difference (abstract). Specifically, See teaches a method of identifying a nucleotide in at least a first position, namely a SNP; hybridizing an oligonucleotide to the target

sequence; wherein the oligonucleotide comprising nucleotides immediately adjacent to the first position, a terminal nucleotide and a florescent label; performing a extension reaction with polymerase extension reagents; monitoring a fluorescent signal; and identifying the first position. Specifically, See teaches a method which depends on pre-amplification and the use of two differently labeled allele specific primers per locus, each of which matches one of two possible allele state in the 3' nucleotide (page 710, col. 3)(limitations of Claim 2). Following initial amplification, labeled internal primers were added to the amplification mixture along with fresh Taq DNA polymerase, dNTPs and buffer (limitations of Claim 4, 8-9). The fluorescent products were amplified and then resolved on gels for detection (page 711, col. 1). The method characterizes the products both by size and by fluorescent label which permits efficient multilocus analysis (page 711, col. 1)(limitations of Claim 12-13). As seen in Table 1, the internal SNP primers each contain a fluorescent dye on the 5' end of the primer (limitations of Claim 5). With respect to claim 5, as written the claim requires that the first fluorescent label is coupled to the first terminal nucleotide. See teaches coupling the label to the 5' end which is a terminal nucleotide. The internal primers are 20 nucleotides in length (limitations of Claim 6-7).

Neither Wallace nor See specifically teaches labeling the 3'-terminal nucleotide.

However, Markiewicz teaches labeling base residues of 3'-terminal nucleosides for sequencing reactions. Markiewicz specifically analyzes whether a 3' end modified nucleoside can be a substrate for DNA polymerases (page 3672, col. 2). The analysis of sequencing reactions with the ALF DNA sequencer from Pharmacia showed

unequivocally that the primer with the 3' end labeled nucleoside unit carrying fluorescein label performed perfectly well in DNA sequencing (page 3673, col. 2). The performance of 3'-end labeled primer was as good as that of the primer RP labeled at the 5'-end with the fluoresceinated Amino link residue (page 3673, col. 2).

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the 5'-end labeled primers, taught by Wallace or See, with 3'-end labeled primers, as taught by Markiewicz. Markiewicz specifically teaches the equivalence of a 3' and a 5'-end labeled primer in a sequencing reaction. Markiewicz teaches that when performing a comparison of end labeled primers, a 3' and a 5' labeled primer were equally as good. Thus, the ordinary artisan would have recognized, at the time the invention was made, substituting a 5' labeled primer with a 3' labeled primer could be used to detect the presence of a single nucleotide polymorphism on a polyacrylamide gel.

12. Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over Wallace et al. (US Pat. 5,639,611, June 1997) or See et al. (BioTechniques, Vol. 28, No. 4, pages 710-716, April 2000) in view of Kumar et al. (US Pat. 5,908,755, June 1999).

Wallace et al. (herein referred to as Wallace) teaches a method of allele specific polymerase chain reaction. Wallace teaches the method is rapid, non-radioactive method for allele specific detection in which the 3' terminal nucleotide of one of the primers of the primer set forms a match with one allele and a mismatch with the other allele. Wallace exemplifies using primers to the human beta-globin gene. DNA from

individuals containing beta-globin was analyzed. Wallace uses oligonucleotide sequence. The oligonucleotides HB14A and HB14S, which are 14 nucleotides in length, differ only in the final 3' nucleotides, such that the oligonucleotides contain a subsequence and a terminal nucleotide which is complementary to one of the possible variant nucleotides (limitations of Claim 2, 6-7). Wallace teaches using labeled oligonucleotides. One of the oligonucleotide primers is labeled at the 5' end with a fluorescent group and the other primer is labeled with biotin (col. 3, lines 15-20; col. 5, lines 15-25, claim 4). With respect to claim 5, as written the claim requires that the first fluorescent label is coupled to the first terminal nucleotide. Wallace teaches coupling the label to the 5' end which is a terminal nucleotide. The fluorescent group is suitable for measuring the amount of the fragment produced. Wallace teaches performing the reaction with first and second primer sets which are differentially labeled at the 5' end with a different fluorescent group (limitations of Claim 12). The oligonucleotides are contacted and allowed to react in the presence of nucleotides, template and Thermus aquaticus DNA polymerase (col. 4, claim 2)(limitations of Claim 4, 8-9). Wallace teaches that the reaction products are quantified by measuring the fluorescence (col. 6, claim 3). The method allows direct detection without probe hybridization, ligation or restriction enzyme cleavage.

See et al. (herein referred to as See) teaches a method of detecting single nucleotide polymorphisms (SNPs) using primers labeled with fluorescent dyes to rapidly and accurately differentiate among alleles that are defined by a single nucleotide difference (abstract). Specifically, See teaches a method of identifying a nucleotide in

at least a first position, namely a SNP; hybridizing an oligonucleotide to the target sequence; wherein the oligonucleotide comprising nucleotides immediately adjacent to the first position, a terminal nucleotide and a florescent label; performing a extension reaction with polymerase extension reagents; monitoring a fluorescent signal; and identifying the first position. Specifically, See teaches a method which depends on pre-amplification and the use of two differently labeled allele specific primers per locus, each of which matches one of two possible allele state in the 3' nucleotide (page 710, col. 3)(limitations of Claim 2). Following initial amplification, labeled internal primers were added to the amplification mixture along with fresh Taq DNA polymerase, dNTPs and buffer (limitations of Claim 4, 8-9). The fluorescent products were amplified and then resolved on gels for detection (page 711, col. 1). The method characterizes the products both by size and by fluorescent label which permits efficient multilocus analysis (page 711, col. 1)(limitations of Claim 12-13). As seen in Table 1, the internal SNP primers each contain a fluorescent dye on the 5' end of the primer (limitations of Claim 5). With respect to claim 5, as written the claim requires that the first fluorescent label is coupled to the first terminal nucleotide. See teaches coupling the label to the 5' end which is a terminal nucleotide. The internal primers are 20 nucleotides in length (limitations of Claim 6-7).

Neither Wallace nor See specifically teaches performing the contacting step in a channel of a microfluidic device.

However, Kumar et al. (herein referred to as Kumar) teaches methods using a microfluidics-based device can be used in sequencing methods for automatedly moving

fluids in and out of a reaction chamber. Kumar teaches methods which combine microfluidics based systems and sequential step sequencing are an attractive alternative to known conventional methods of nucleotide sequencing. The use of the microfluidic device eliminates the need for electrophoresis, which is one of the most time-consuming steps of the sequencing reactions of the priori art (col. 2, lines 15-23).

Therefore, it would have been prima facie obvious to one of ordinary skill at the time the invention was made to have modified the electrophoresis based detection assays of Wallace or See with the teachings of Kumar. Kumar teaches that microfluidic based methods eliminates the need for electrophoresis, which is one of the most time-consuming steps of the sequencing reactions of the priori art (col. 2, lines 15-23). Thus, the ordinary artisan would have been motivated to use a microfluidic device for performing the sequencing methods of See or Wallace. The ordinary artisan would have desired to use methods which are less time consuming and automated to detect nucleic acid sequences. Performing a more efficient method for detecting sequencing variations would have been attractable to the ordinary artisan. Therefore, substituting the more efficient method of detecting sequences using a microfluidic device in place of a more time consuming detection means of electrophoresis would have been obvious at the time the invention was made.

Allowable Subject Matter

13. The instant specification discloses using a 3'-end labeled oligonucleotide to perform primer extension and monitoring the level of polarized fluorescence emitted

from the extension reaction wherein a decreased in polarized fluorescence indicates the presence of polymerase extension. The prior art teaches fluorescence polarization (FP) is directly proportional to molecular weight (Kwok et al. US Pat. 6,180,408, January 2001). Thus, the ordinary artisan would expect with the increase in nucleotides, the fluorescence polarization would also increase. Moreover, Kwok teaches using a sequencing primer which is unmodified with its 3'-end immediately upstream from the polymorphic site. Kwok does not teach 3'-terminal labeling of the oligonucleotides or labeling the primer prior to extension.

However, given the examples provided in the instant specification, a perfectly matched probe to the target sequence shows a decrease in polarization (Figure 5A). Alternatively, the mismatched probe/target illustrates no decrease in polarization. The specification reports this as surprising (page 6, lines 15-20). The instant specification also reports that as the labeled probe is extended, the homogeneous reaction mixture has increased fluorescent intensity. Therefore, since the detection of a decreased in polarized fluorescence of a 3'-end labeled oligonucleotide shows an unexpected decreased in polarized fluorescence, claims drawn to a method of identifying a nucleotide at in at least a first position using a 3'-terminal nucleotide labeled probe and measuring a level of polarized fluorescence emitted from an extension reaction wherein a decreased in polarized fluorescence indicates the presence of polymerase extension appears free of art.

Conclusion

14. No claims allowable.

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner Jeanine Goldberg whose telephone number is (703) 306-5817. The examiner can normally be reached Monday-Friday from 8:00 a.m. to 5:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Jones, can be reached on (703) 308-1152. The fax number for this Group is (703) 305- 3014.

Any inquiry of a general nature should be directed to the Group receptionist whose telephone number is (703) 308-0196.

J. Goldberg

Jeanine Goldberg
Patent Examiner
August 7, 2003